

BRMAC briefing document for Day 2 November 17, 2000

Follow-up of Subjects in Gene Transfer Clinical Trials

Session IV. Issues in germ line transmission

Inadvertent germ line transmission remains a theoretical but real concern for gene transfer clinical trials. The risk is particularly applicable in clinical trials where integrating gene transfer vectors are administered by systemic routes. While germ line transmission has not been observed in clinical trials to date, there are preclinical data suggesting this is more than a theoretical risk. For example, data from one study in rats wherein a retroviral vector was injected intracardiac showed evidence of gene transfer not only in the treated rats but also in their F1 and F2 offspring as well, demonstrating that germ cells were transduced by the retroviral vector [1]. In other cases, germ line gene transfer may only occur with more directed attempts. For example, direct injection of liposome-encapsulated plasmid into mouse testis followed by breeding was successful in transferring the transgene to offspring [2]. The relevance of these kinds of reports is less obvious, but they support the conclusion that the risk is real.

Data on the risks associated with germ line gene transfer are available from studies performed with retroviruses, where these viruses were in fact used as a tool to derive mouse strains with recessive lethal mutations induced by provirus mutations (for review, see [3]). To assess the mutagenic effect of retrovirus insertion, heterozygous animals were intercrossed in order to obtain homozygous animals at each locus. Combining results from a number of studies, two out of 48 crosses produced recessive lethal mutations [4].

Discussions with the Recombinant DNA Advisory Committee

On March 12, 1999, the Recombinant DNA Advisory Committee (RAC) met to discuss gonadal biodistribution studies and potential risk of inadvertent germ line transfer (see <http://www4.od.nih.gov/oba/3-99RAC.htm>). In summary, although the risk of inadvertent germ line transmission was considered low, the RAC recommended platform studies for certain classes of viral vectors to support human studies.

FDA's current recommendations for biodistribution studies:

Animal Treatment

- Route of administration should mimic intended clinical use as closely as possible
- Inclusion of vehicle control, at least two doses of test article (to establish NOEL for biodistribution, and to attempt to maximize exposure)
- Sacrifice/sampling times should include at least one time point where gene expression is at peak, and one later time point

- If integrating vector, should include time point of at least 60 days, to determine potential for signal persistence through one complete cycle of spermatogenesis
- If planning on repeat administrations for the clinical trial, later time point should be at approximately the time of vector re-administration planned in humans
- Whenever feasible, peripheral blood should be removed from tissue samples as much as possible at necropsy

PCR Assay Conduct

- Assay should detect a minimum of 100 copies of vector signal per mcg of DNA
- Minimum of 3 mcg of DNA per tissue should be assayed
- Two mcg of DNA per tissue sample to be assayed directly, with no further treatment
- One mcg of DNA per tissue to be “spiked” with positive control for vector amplification, as control for effects of tissue factors on detection of signal

Patient follow up

FDA has not developed specific recommendations regarding semen analysis or other tissue analysis to assess germ line dissemination. Most retroviral vectors are administered *ex vivo*. The requirements for patient testing (which does not include semen analysis) are outlined in section III. In the one ongoing clinical study of direct IV injection of a retroviral vector, semen samples are obtained at time 0, week 2,4,6,17,29, and 52 weeks. Study participants are to use barrier contraception for one year after vector administration. Recently a patient sample was positive by PCR, and the study was put on clinical hold until 3 consecutive semen samples were negative. In another ongoing study of 3 direct (IM) administrations of an AAV vector, semen analysis is performed at time 0, 1,2, and 3 months. As above, any positive sample will lead to a clinical hold until 3 consecutive samples are negative. The potential for gonadal dissemination is included in informed consent documents.

References:

1. Reaves, P.Y., et al., *Permanent cardiovascular protection from hypertension by the AT1 receptor antisense gene therapy in hypertensive rat offspring*. Circulation Research, 1999. **85**: p. e44-e50.
2. Sato, M., et al., *Testis-mediated gene transfer (TMGT) in mice: successful transmission of introduced DNA from F0 to F2 generations*. 1999.
3. Jaenisch, R., et al., *Retroviruses and insertional mutagenesis*. Cold Spring Harbor Symposia on Quantitative Biology., 1985. **50**: p. 439-445.
4. Soriano, P., T. Gridley, and R. Jaenisch, *Retroviruses and insertional mutagenesis in mice: proviral integration at the Mov34 locus leads to early embryonic death*. Genes and Development, 1987. **1**: p. 366-375.

DRAFT Questions to the committee

1. If semen positivity is identified in patients in a clinical trial, our current approach is to place a clinical hold on the study until it is determined that semen positivity is transient, at which time, the trial may be resumed. Please discuss whether this approach is appropriate.
2. In cases where a positive PCR signal is observed in patient semen samples, please discuss methods to determine cell source for the positive signal (e.g., in situ hybridization, fractionation of semen into sperm and lymphocytes, etc.).